Part 1 – Practice

1. **Sequence alignment hands-on exercise.** You are given the following protein sequence:

   ```
   >Protein
   TCPFADPAALYSRQDTTSGQSPLAAYEVDDSTGYNSTGVGGPIQDQTSLKAGIRGPTLEDQFMRQIQHFDHERVPERAV
   ```

   (a) Go to the Blast website at http://blast.ncbi.nlm.nih.gov. Under “basic Blast” select “Protein Blast” to get to the BlastP website. Paste the above sequence (including the line begins with >Protein) to the top window. Use the default parameters – nr database (non-redundant protein sequence database). Hit “BLAST” – the search may take a few seconds. Save the search results using the “download” button and the “txt” option above. Submit a printout of the first 2 pages of the saved search as part of your homework. **DO NOT print out the entire search result – it’s very large.**

   (b) Repeat the search above with the SwissProt database and submit its first 2 pages as part of your homework.

   (c) Repeat search a. above with PAM30 as a substitution matrix. This can be done in the blastP homepage by opening “algorithm parameters” at the bottom of the page. Observe the changes between the results of a and c due to the change in the substitution matrix: Look at the first entry that differs between a and c. What is its rank in a and c? What is the name of the protein sequence in this entry? The results may change from one year to another. The first different entry was 4AUE which was first in BLOSUM62 but sixth when PAM30 was used.

2. **DNA sequence alignment:** The following sequence was constructed by NCBI scientist Mark Boguski for Michael Chrichton’s “The Lost World” of the Jurassic Park series:

   ```
   >DinoDNA from THE LOST WORLD p. 135
   GAATTCGGAGAGCAAGAGATAAGTCTGGCAGACGATAGTGGAGATAGACG
   GACGTGTGGCAGCTCCCCGCAGAGGATTCACCTGGAACATTGCACTACCTTATCCCCATGGGAGCC
   ATGGAGTTCTGTGCGCTGAGCTGGGGAGGGCCGATGCGGCTCCCCACTCTGCTGGCTATGAA
   GCCGGAGCTCTTCTGGGCTGGAAGGGGAGCAGGCAGGGGGGGGCTGCTGGCC
   TCCTACCCCCCCTCAGGCGCTGCTCTGCTGCGCTGGGAGACAGACGCTTGGG
   ACCCAGAGGTGGCTGCGCCGCCGCCGCCACCCAAATGGAGCCTCCCACTACTGGAGCTTGCTG
   CAACCCCGGGGAGCGCCACCCCATCTCCTCAGGCCCCCTCTCGCACTCGACGC
   GGGCCCAACCTGCGAGCGGCTGGAATGCTGCTGCGCTGGACGAACTGGGAGAGC
   GCAACGCGCTGTGCGCAGCGACGACGGAATTACCTGTGAACATTGCGCTACGG
   TGGGGCTCTACCAACCGCTCAAGCGCGCAAGACCCGCGCTCATCCGCCCCAAGAAGGCG
   CTGCCTGCTGAGTAAGCGCGACGCAAGCTGCTGCAACGCGAGGGTAGAATCTGGCAACAGCA
   TCCACGCAACACTCTGCTGGGAGCGCCCTCAGGGAGGAGCAGCCGCTGCGCTG
   GGAATCCAAAACCCGAGAACGCGAGTTTCTCTCAGGGAAGGTAAGCGCAGCCCGGCGG
   GGGGAAACCCCTCCGACGCCGAGGGGCCTATGGGAGGAGGGGACCC
   ```
Perform a Blast search using blastn (nucleotide search) and the default non-redundant (nr) nucleotide database.

(a) What are the two main species used to construct the dinosaur DNA sequence?
Frog and chicken

(b) Repeat the search with blastx (DNA vs. protein sequence) using the default non-redundant protein sequence database. Look at the top sequence alignment and retrieve the hidden message there (hint: look at the gaps...).

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(Mark Boguski was obviously playing a little practical joke on the way).

**Part 2 – Theory**

(a) Lesk book question 5.3: The edit distance between the strings agtcc and cgctca is 3, consistent with the following alignment:

\[
\begin{align*}
\text{agtcc} & \quad \text{cgctca} \\
\text{ag} & \quad \text{c} \\
\text{tcc} & \quad \text{gctca}
\end{align*}
\]

Find the sequence of three edit operations that convert agtcc to cgctca.

- Substitution \( a \rightarrow c \) at first position
- Insertion (c after g)
- Substitution \( c \rightarrow a \) at last position

(b) **Dynamic programming:**

i. Use the Needleman Wunsch global alignment Dynamic programming formula in slide set no. 2 to find the sequence alignment score of the two DNA sequences TACGGGTAT and GGACGTACG. Show the filled dynamic programming matrix using +1 for a match, -1 for a mismatch and -1 for a gap penalty in a way similar to the slide sets.
ii. Repeat with the Smith-Waterman local alignment algorithm and the same scoring scheme.

(c) Substitution matrices:

i. Given the BLOSUM-62 matrix (see sequence class notes or http://www.ncbi.nlm.nih.gov/IEB/ToolBox/C_DOC/lxr/source/data/BLOSUM62), find the score of the following alignment (assume this is the optimal alignment):

\[ \text{THISSEQ} \]
\[ \text{THATSEQ} \]

\[ 5+8-1+1+4+5+5=27 \]

ii. Repeat with the PAM-250 matrix. It can be found in Lesk, page 257, or here: http://www.ncbi.nlm.nih.gov/IEB/ToolBox/C_DOC/lxr/source/data/PAM250

\[ 3+6-1+1+2+4+4=19 \]

(d) Multiple Sequence Alignment: Extend the dynamic programming formula to 3 dimensions. What is the run time in this case? How many cases do we have to compare this time?

Hint: this time the matrix is cubic since instead of a 2-dimensional matrix we need to run on a cube of \( m \times n \times k \) where \( m, n, \) and \( k \) are the lengths of the three sequences. Every path goes from one vertex of the cube and traveling inside the cube to another vertex. Try to count how many such paths there can be.
The run time is cubic, $O(m \times n \times k)$ because you have to fill out a 3-D matrix instead of a 2-D, going over all the possible positions. However, what makes the problem difficult is not only the increased number of sequences, but the number of neighbors we have to compare for every step. This time we have 7 instead of 3 (think of the neighbors that precede a given position). In other words, the number of neighbors increases exponentially with the dimension, which hints to why the problem becomes NP-hard in higher dimensions.